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NOTES ON GEOGRAPHIC DISTRIBUTION

Entoloma virescens (Sacc.) E. Horak ex Courtec., 1986 (Agaricales: Entolomataceae): The first record for the Caatinga biome, Ceará, Brazil

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ABSTRACT: *Entoloma virescens*, a rare mushroom with deep blue coloring and cuboid spores, is recorded for the first time in the Caatinga biome in Brazil. The relevance of this finding is that such area is characterized by an atypical environment for the occurrence of species normally considered from temperate and humid tropical regions. The record is fully described and illustrated. Also, a brief analysis of taxonomical features and geographical distribution of the taxon are included.

The family Entolomataceae (Basidiomycota, Agaricales) Kotl. and Pouzar is very rich in species, comprising over 1500 recognized taxa that occur worldwide from tropical to arctic habitats (Largent 1994; Noordeloos and Gates 2007; Noordeloos and Hausknecht 2007; Noordeloos and Gates 2009). According to Co-David *et al.* (2009) this family traditionally contains three main genera: *Clitopilus* (Fr. ex Rabenh.) P. Kumm., *Rhodocybe* Maire and *Entoloma* (Fr.) P. Kumm. *sensu lato*. Though these authors commented that the latter genus is a monophyletic group that has high diversity in morphological features and is considered the second largest genus within the Agaricales.

The genus *Entoloma* has been studied by Noordeloos on a worldwide scale (Noordeloos 1980, 1981; Manimohan *et al.* 2006; Noordeloos and Gates 2007; Noordeloos and Hausknecht 2007; Noordeloos and Gates 2009; Noordeloos and Morozova 2010). However, data are still lacking on occurrence and distribution of many species in South America, in particular from the Brazilian territory. To date, few species have been reported and only about 56 taxa of *Entoloma* are recognized (Putzke and Putzke 2000). In the Northeast region, this number drops dramatically since the majority of taxonomic studies on *Agaricales* are concentrated in the south and southeast of the country. Hence, very few taxa of *Entoloma* have been described for the Brazilian Northeast (Singer 1965; 1973; Horak 1977; 1982; Wartchow 2006).

The present study deals with *Entoloma virescens*, a rare mushroom with strong blue color and cuboid spores, which is recorded for the first time in the Caatinga biome of Brazil. The latter area is considered an atypical environment for the occurrence of the species, which, to the best of our knowledge, has only been recorded in forested areas of temperate and humid tropical regions.

The specimen under consideration was collected during forays in the area of Bom Gosto, Pacujá municipality, Ceará state, approximately 248 km from the capital Fortaleza. The area has an average elevation of 160 m a.s.l.; it is irrigated by a number of water courses and is characterized by warm sub-humid climate with a rainy season occurring between the months of January and April. In terms of physiognomic attributes, the region is an area of vegetation types associated with the warm tropical semiarid climate, within which the highest percentage of coverage is represented by the open shrubby Caatinga and dense shrubby Caatinga (Figueiredo 1986; Fernandes and Bezerra 1999).

Field collections took place in March 2011. Specimens were collected, documented and preserved using standard methods (Largent *et al.* 1986). The material was photographed in the field using a digital camera and extensive notes on the basidioma were made before drying. Microscopic analysis of the material was performed using an Olympus BX41 microscope.

All measurements and colors reported for microscopic features were made from dried material rehydrated in 96% ethanol followed by distilled water, distilled water + blue cotton, 3% KOH or Melzer's reagent. At least, 25 measurements were made of each microstructure. Measurements of cuboid spores were made in profile view and the apiculus was not considered. Spore statistics include arithmetic means (x_m) of basidiospore lengths and widths ± standard deviation measured for n objects; quotient of basidiospore length by spore width (E) indicated as a range variation in n objects measured; the mean of E-values (Q) ± standard deviations. The sample size (n) = total number of basidiospores measured (x) divided by the number of basidiomata studied (y), as shown in the formula n = x/y (Largent and Abell-Davis 2011).

Digitized microphotographs were taken using a camera attached to a compound light microscope. The material was identified by comparing macro and microscopic features with previous studies of Romagnesi (1941), Horak (1976), Pegler (1986), Noordeloos and Hausknecht (2007) and Largent and Abell-Davis (2011). In addition,

the terminology of Kirk *et al.* (2008) was followed. The documented material has been deposited at the Herbarium Graziela Barroso (TEPB) of the Universidade Federal do Piauí (UFPI).

Description of Material Collected - ***Entoloma virescens*** (Sacc.) E. Horak ex Courtec., Mycotaxon 27: 131. 1986. (= ***Agaricus virescens*** Berk. and M.A. Curtis, Proc. Amer. Acad. Arts 4: 116. 1860, nom. Illegit. (non Schaeff. 1774); = ***Inocephalus virescens*** (Sacc.) Largent and Abell-Davis, Mycotaxon 116: 232. 2011; = ***Inopilus virescens*** (Sacc.) Pegler, Kew Bull. Add. Ser. 12: 268.1986; = ***Leptonia virescens*** Sacc., Syll. Fung. 4: 714. 1887). **Figure 1 and 2**.

Pileus 3.0–4.5 cm diam., 3.5–4.5 cm high, conic-convex with a papillate umbo, then occasionally depressed at center; slightly hygrophanous, translucently striate when moist, becoming fibrillose near the wavy margin; surface at first deep blue becoming greenish-blue to entirely grayish-green when mature or drying, and staining yellow-blue greenish in place when pressed, cut or bruised. **Lamellae** adnexed, moderately spaced, with lamellulae of three lengths, ventricose, dull blue becoming a yellowish-green or greenish-blue on exposure or handling; margin smooth to slightly serrated. **Stipe** 4.5–5.5 cm × 5–7 mm, central, confluent with the pileus, fragile, cylindrical, flattened, hollow, glabrous, slightly striated; concolorous with the pileus, with white basal mycelium. **Context** thin, dull blue becoming dark green on exposure or drying, consisting of hyaline, thin-walled, inflated hyphae. **Odor** distinct but unidentifiable. **Taste** unknown.

Basidiospores 9.75–13.75 x 9.75–12.5 µm (x_m = 11.72 ± 1.16 x 11.29 ± 0.84; E = 0.90–1.16 (-1.65); Q = 1.06 ± 0.136; n = 30/2), cuboid, with four regular to irregular angles in profile or side views, slightly yellowish in 3% KOH, with a slightly thickened, stramineous wall. **Basidia** 32–50 × 14–18 µm, 4-spored, clavate to subcylindrical, thin-walled, granular, with brownish to stramineous vacuolar contents. **Lamelar edge** with pseudocystidia and cheilocystidia present. **Pseudocystidia** of tramal origin, sinuous, slightly constricted-lobed, rounded apex, thin-walled, present at the edges and sides of the lamellae, 6–9.2 µm diam. **Cheilocystidia** 28–55.5 × 11–16.75 µm, cylindro-clavate, yellowish in 3% KOH with golden brown vacuolar contents, thin-walled. **Hymenophoral trama** regular, composed of cylindrical, hyaline, thin-walled, inflated hyphae, 6–12.5 µm diam., with brownish vacuolar contents; abundant vascular hyphae. **Subhymenial layer** large, 62.5– 100 µm wide, with abundant vascular hyphae. **Pileipellis** a cutis of hyaline, cylindrical, repent hyphae, 5.75–13.5 µm diam., with abundant stramineous intracellular granules. **Clamp connections** common in all tissues. **Habit and habitat**: Solitary or disperse growing on the soil in shady area, collected in open shrubby Caatinga zone during the rainy season. **Material examined**: **BRAZIL**. **Ceará State**: Pacujá, Community of Bom Gosto, 16-III-2011, collectors: Alves, M. H and Nascimento, C. C. 072/11 (TEPB) (02°55'49.5" S; 41°40'34.1" W; Elev. 140 m a.s.l.).

Morphologically *E. virescens* can be recognized by the following combination of characters: basidioma at first

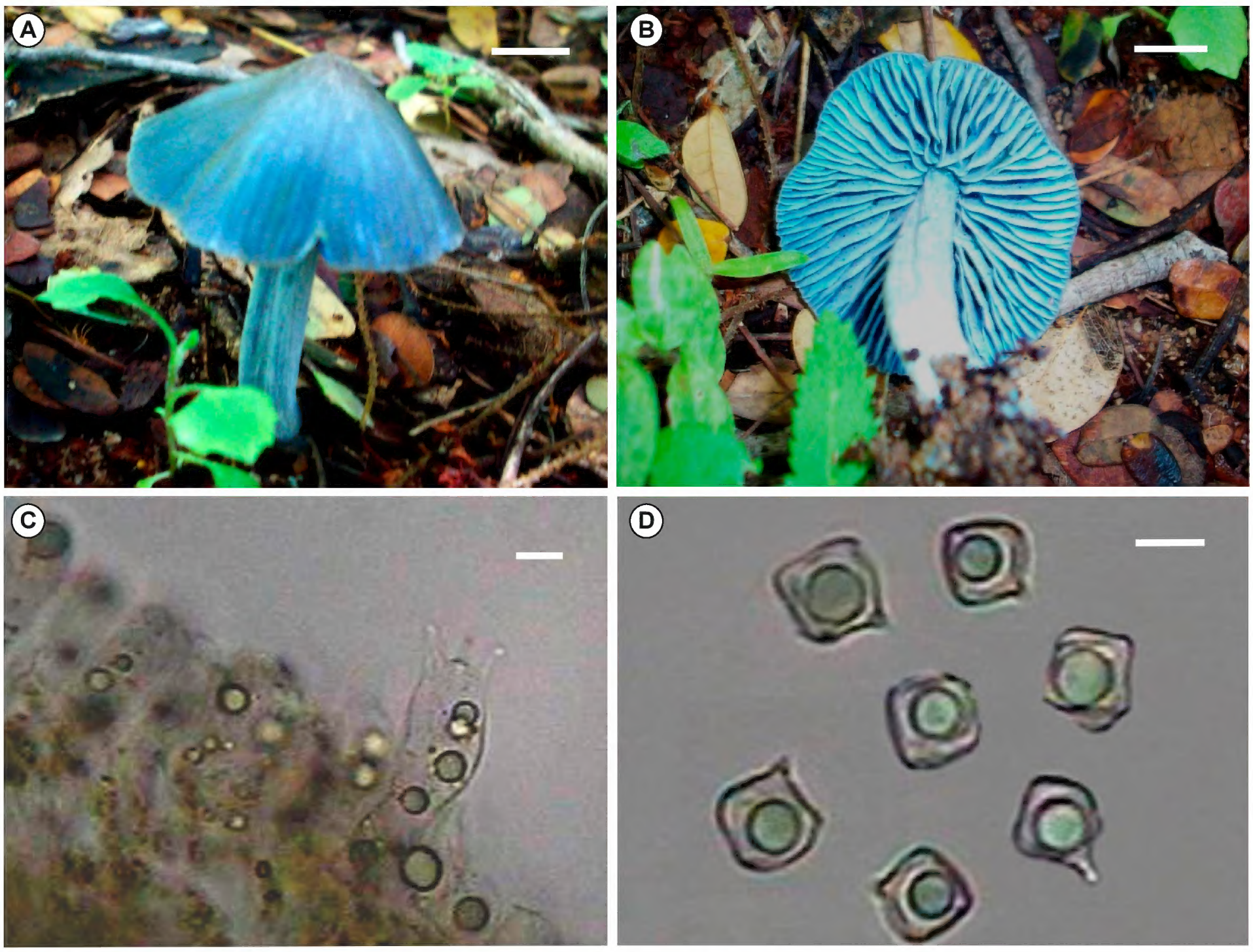

Figure 1. *Entoloma virescens*. A and B: Basidioma; C: Basidium and basidioles; D: Basidiospores. Bars: A and B = 10 mm; C and D = 10 µm. Photos A and B: Maria Helena Alves; C and D: Cristiano Coelho do Nascimento.

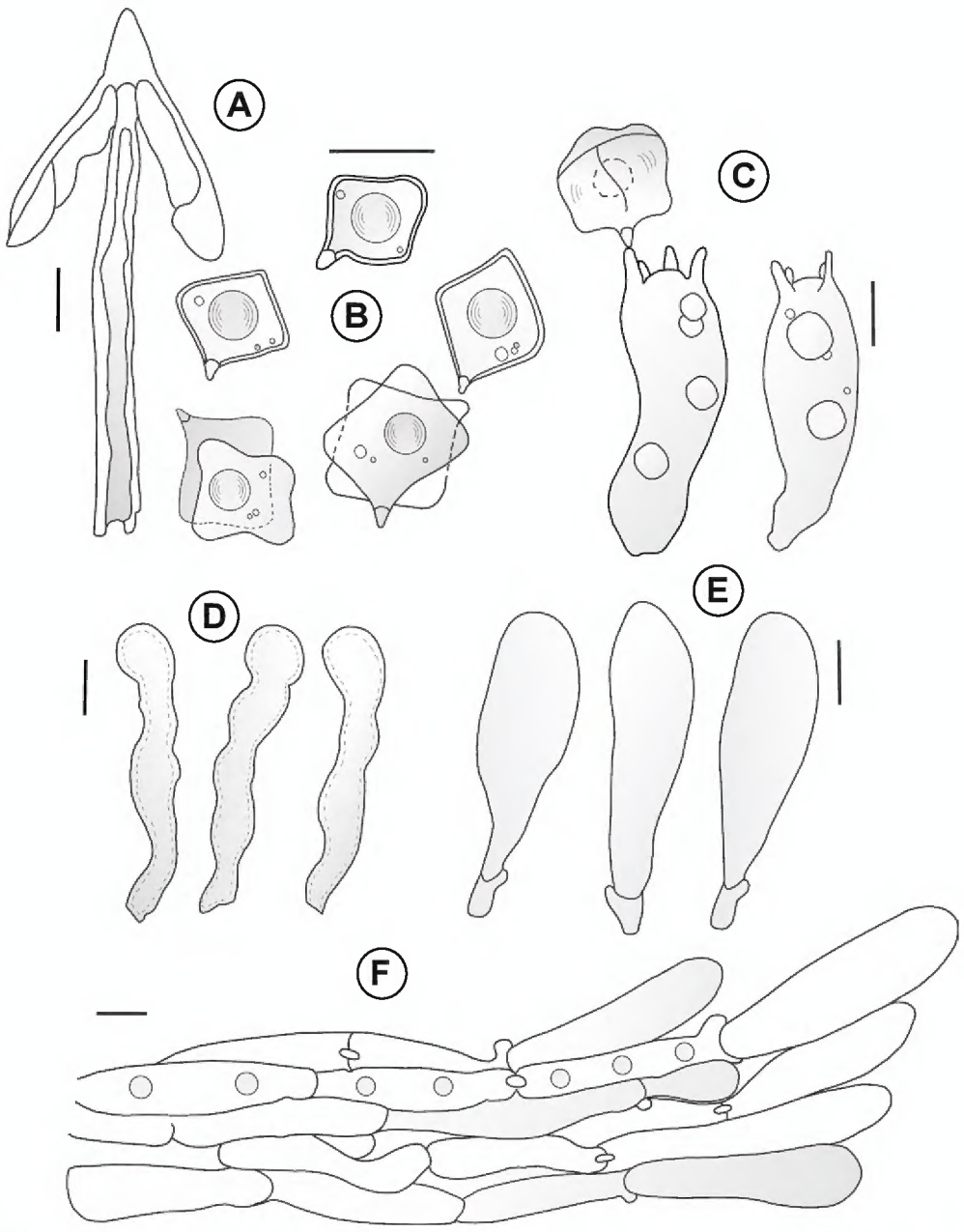

Figure 2. Entoloma virescens: A, basidioma; B, spores; C, basidia; D, pseudocystidia; E, cheilocystidia; F, pileipellis. Bars = 10 mm (basidioma) and 10 µm (microscopic characters).

deep blue becoming greenish-blue to entirely grayish-green when mature or drying, and staining yellow-blue greenish in places when pressed, cut or bruised; conico-convex pileus with a papillate umbo; strictly 4-angled basidiospores; cheilocystidia cylindro-clavate; pseudocystidia of tramal origin; vascular hyphae very abundant and clamp connections common in all tissues.

This species was the first record of *Entoloma* with cuboid spores published in 1857, when Berkeley and Curtis described it for Bonin Island, however, these authors did not recognize yet the peculiar shape of the spores. Only 80 years later, Romagnesi (1941), studying parts of the collection type kept in Paris, discovered the particular cuboid spores (Horak 1976). Examining the publication above mentioned, Romagnesi (1941) "Les Rhodophylles de Madagascar", and using the key contained in this work, the set of features of the material examined in this study lead to the species *Rhodophyllus holocyaneus* Romagn. in the *Psittacine* section, which is also observed in the material of Noordeloos and Hausknecht (2007) from La Réunion. The referred species is characterized by the greenish-blue color of the basidioma, conical pileus, cuboid spores and abundant pseudocystidia originated from the tramal vascular hyphae that reach the hymenial layer. The latter feature is strongly evident in the material examined from Pacujá/CE, Brazil, differing from the material described by Noordeloos and Hausknecht (2007) as *E. virescens sensu lato*, in which the presence of pseudocystidia from tramal origin is not cited. Considering the taxonomic and nomenclatural changes presented by Co-David *et al.* (2009), which deal with the phylogenetic reconstruction of the family *Entolomataceae*; *R. holocyaneus* becomes the basionym of the new combination *E. holocyaneum* (Romagn.) Noordel. and Co-David.

Horak (1976) considered *R. holocyaneus* together with *E. hochstetteri* (Reichardt) G. Stev. as well as *E. aeruginosum* Hiroë (species found in Japan) as being conspecific with *A. virescens* from the Bonin Islands, proposing a new combination: *E. virescens* (Berk. and M.A Curt.) E. Horak, but without referring the full citation of the basionym. Later, Coutecuisse (1986) validated the combination. However, Berkeley and Curtis authority is an illegitimate name since the epithet '*virescens*' was already used by Schaffer in 1774 (Largent and Abell-Davis 2011). Romagnesi (1941), who studied the type collection preserved at Kew Herbarium emphasizes that *A. virescens* has a conical pileus, differing from the center-depressed pileus that is specified in the diagnosis of Berkeley and Curtis.

Hongo (1990), differing from Horak (1976), prefers to consider *E. hochstetteri* and *E. aeruginosum* as separate taxa, due to the differences in the spore size and shape of the pseudocystidia. *Entoloma aeruginosum* has spores measuring 10.5–12.5 x 9.5–12 µm and pseudocystidia subcylindrical to clavate; while *E. hochstetteri* basidospores measure 12.5–5.5 × 12–15 µm and presents pseudocystidia subfusoid to subclavate. In these aspects, our specimens of *E. virescens* collected in Pacujá/CE, Brazil, have spores measuring 9.75–13.75 x 9.75–12.5 µm and sinuous pseudocystidia, constricted-lobed, differing in relation to the two taxa mentioned above.

Courtecuisse (1986), who examined two collections of *E. aeruginosum*, found some morpho-anatomical features differing from those observed in the analysis of *A. virescens* holotype and the descriptions made by Romagnesi (1941) for *R. holocyaneus*. The author emphasized simple cuboid spores of the type *A. virescens* in contrast to the complex shape of the spores of *E. aeruginosum*, also highlighting differences in the subhymenial layer between *R. holocyaneus* and *E. aeruginosum*. In this manner, it seems that the correspondence of *E. aeruginosum* with *A. virescens* and *R. holocyaneus* is not absolutely perfect; however, Courtecuisse (1986), in possession of restricted taxonomic data, corroborated the terms proposed by Horak (1976), highlighting the inaccuracy of some synonymies.

Pegler (1986) described *Inopilus virescens* (Berk. and Curt.) Pegler, comb. inval. for the central province of Sri Lanka as a distinct species in young stages by virtue of the uniformly blue coloration of the basidioma with the blue tints disappearing at maturity. In this new combination, Pegler defined *A. virescens* as basionym, putting *E. virescens* under synonymy. Pegler (1986) recongnized *R. chloroconus* Romagn. and Gilles from Ivory Coast as a species closely related to *I. virescens*, although the first species lacks the blue color, and the cuboid spores have strongly depressed facets. *Inopilus virescens* was also recorded for South America by Pegler (1997); being described as a species with cuboid spores [10– 12(-14) x 9–11µm], quadratic in profile, lamellae deep blue; basidioma at first pale to deep blue becoming yellowish green; pleurocystidia 40–120 µm long, cylindrical to ventricose, with brown vascular pigment.

In the record for Campos do Jordão/SP, Brazil, Pegler (1997) cited the occurrence of robust pleurocystidia; however this feature was not reported by Pegler (1986)

for *I. virescens* and was not observed in the material recorded for the Brazilian Northeast. With regard to spore dimensions, values measured in the material examined from Pacujá/CE (9.75–13.75 x 10–12.5 µm) are closer to those cited by Pegler (1997) (10–12(-14) × 9–11 µm) for Campos do Jordão/SP, differing from the basidiospores (8.5–11 × 8–10.5 µm) observed by Pegler (1986) in the material from Sri Lanka that are slightly smaller than those mentioned above.

Based on recent examination of several collections from Northeastern Queensland and New South Wales (Australia), Largent and Abell-Davis (2011) proposed the new combination, *Inocephalus virescens,* for *E. virescens.* Our collections from the Brazilian Northeast, for the most part, match the description of *I. virescens*; however, the 4-5-angled basidiospores and cylindro-clavate pileocystidia and caulocystidia differentiate *I. virescens sensu* Largent and Abell-Davis from our material, which lacks cylindro-clavate pileocystidia and caulocystidia and all basidiospores are strictly 4-angled. Therefore, it seems that the correspondence of our material with *I. virescens sensu* Largent and Abell-Davis is not well supported. In this manner, based on morphological similarity, we consider that *E. virescens sensu* Noordeloos and Hausknecht are more closely related with our findings.

Regarding the distribution, Horak (1976) considered the region between Malaysia and New Zealand as being the center of dispersion for *Entoloma* species with cuboid spores, assuming the occurrence of numerous taxa for the African continent. The author also showed that these species occur mainly in the tropical and subtropical range, with many species also occurring in forests in temperate zones.

With respect to *E. virescens* in the wide sense, it has been characterized as a widely distributed species, being reported by Horak (1976) for Bonin Island (type), New Zealand, Japan, Malaysia, Madagascar, Papua New Guinea and Sri Lanka. Pegler (1986) added collections from Zambia and Thailand. In all of these reports the species is cited occurring mainly in the soil of tropical and subtropical forests. In South America, *E. virescens* is cited by Pegler (1997) for the municipality of Campos do Jordão/SP, Brazil, which is characterized by altitudinal tropical climate and by vegetation constituting of a typical field and forest mosaic.

The record herein presented registers *E. virescens* for the first time for the Caatinga biome in the Brazilian Northeast. Such area represents an atypical environment according to physiognomic aspects of the environments mentioned thus far for the taxon, showing that the species is widely distributed and able to occur in different habitats without showing large morphological changes.

We conclude, as did Noordeloos and Hausknecht (2007) for their material, that the taxon with cuboid basidiospores and blue-colored basidiomata from the Brazilian Northeast fits the concept of *E. virescens sensu lato* and occurs within a very wide geographical range. However, the clarification and elucidation of the *E. virescens* complex is very complicated when highly diverse morphological characters alone have not been enough to establish specific limits due to the absence or poor condition of many holotype collections (Largent and Abell-Davis 2011). Therefore, we consider that molecular sequence analyses would help to interpret the significance of morphological characters helping to establish the species limits and its geographical range.

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